Search Strategy

FILE 'USPATFULL' ENTERED AT 18:07:50 ON 03 MAR 2003

		E	BENVENISTE RAOUL E/IN
L1	2	S	E3
		E	SHEARER GENE M/IN
L2	6	S	E3
L3	5	S	L2 NOT L1
		E	CLERICI MARIO S/IN
L4	2	S	E3
L5	1	S	L4 NOT (L1 OR L2)
L6	21817	S	(HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L7	1769	S	L6 AND (NC OR NUCLEOCAPSID OR P7)
L8	148	S	L7 AND (NC/CLM OR NUCLEOCAPSID/CLM OR P7/CLM)
L9	49	S	L8 AND (REPLICATION-IMPAIRED OR REPLICATION-DEFICIENT OR REPL
		E	ROVINSKI BENJAMIN/IN
L10	21	S	E3
L11	19	S	L10 AND (GAG OR NUCLEOCAPIS OR NC OR P7)
L12	0	S	L11 AND (NC OR NUCLEOCAPSID OR P7)
L13	5	S	LENTIVIRAL EXPRESSION VECTOR?
L14	186	S	EIAV
L15	116	S	L14 AND (EXPRESSION VECTOR?)
L16	96	S	L15 AND (GAG OR NC OR P7 OR NUCLEOCAPSID)
L17	52	S	L16 AND VECTOR/CLM

FILE 'WPIDS' ENTERED AT 18:32:49 ON 03 MAR 2003

		E	BENVENISTE R E/IN
L18	4	S	E2 OR E3
		E	SHEARER G M/IN
L19	10	S	E3
		E	CLERICI M S/IN
L20	3	S	E3
L21	14526	S	(HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L22	122	S	L21 AND (NC OR NUCLEOCAPSID OR P7)
L23	9	S	L22 AND (REPLICATION-IMPAIRED OR REPLICATION-DEFECTIVE OR REP
L24	4	S	(LENTIVIRAL EXPRESSION VECTOR?)

FILE 'MEDLINE' ENTERED AT 21:54:21 ON 03 MAR 2003

		E	BENVENISTE R E/AU
L1	169	S	E3 OR E2
L2	37	S	L1 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L3	4	S	L1 AND (NC OR NUCLEOCAPSID)
		E	SHEARER G M/AU
L4	349	S	E3
L5	0	S	L4 AND (NC OR NUCLEOCAPSID)
L6	0	S	L4 AND P7
L7	111	S	L4 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L8	4	S	L7 AND GAG
L9	19	S	L4 AND VACCINE
		E	CLERICI M S/AU
L10	240	S	E2
L11	0	S	L10 AND (NC OR NUCLEOCAPSID OR P7)
L12	144	S	L10 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

L13	24	S	L12 AND (DEFECT? OR DEFICIENT? OR IMPAIRED)
L14	128464	S	(HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L15	578	S	L14 AND (NC OR NUCLEOCAPSID OR P7)
L16	28	S	L15 AND VACCIN?

L1 ANSWER 1 OF 2 USPATFULL
2001:211940 Method of inducing cell-mediated protective immunity against HIV using low doses of immunogens.
Benveniste, Raoul E., Bethesda, MD, United States
Shearer, Gene M., Bethesda, MD, United States
Clerici, Mario S., Rockville, MD, United States
Secretary of the Department of Health and Human Services (U.S. corporation)
US 2001043932 A1 20011122
APPLICATION: US 2001-769223 A1 20010124 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method and composition is disclosed for inducing a protective immunity against HIV by inoculation with immunogens in low doses that are sufficient to induce a sustained cell-mediated response to HIV but below the amount necessary to induce an offsetting humoral response. The immunogens available for use in this method include attenuated forms of the HIV virus, subunits of the HIV virus, inactivated HIV virus and subinfectious doses of live HIV virus, all delivered at low doses. These immunogens can be prepared with-suitable carriers, adjuvants or diluents and administered either intramuscularly, mucosally (e.g., orally), intravenously or subcutaneously. The effectiveness of the initial dose of immunogen can be monitored for the presence of a sufficient cell-mediated response using a T-cell proliferation assay or an interleukin-2 assay and monitored for the lack of offsetting humoral response using commercially available ELISA assays for anti-HIV antibodies. Depending upon the results of the cell-mediated response assays, supplementary or "booster" inoculations may be appropriate.

CLM What is claimed is:
1. A method for vaccinating a human against a human immunodeficiency virus comprising the steps of: selecting an immunogen competent to induce a protective immune response in said human against said human immunodeficiency virus, and administering to said human an effective amount of said immunogen sufficient to induce a sustained cell mediated immune response against said human immunodeficiency virus.

2. The method of claim 1 wherein said immunogen is an attenuated form of human immunodeficiency virus.

3. The method of claim 2 wherein said immunogen has been attenuated by removing all or part of the nef gene from the nucleic acid of said human immunodeficiency virus.

4. The method of claim 1 wherein said immunogen is a subunit of said human immunodeficiency virus.

5. The method of claim 4 wherein said immunogen is a gp120 subunit of said human immunodeficiency virus.

6. The method of claim 4 wherein said immunogen is a gp160 subunit of said human immunodeficiency virus.

7. The method of claim 1 wherein said immunogen is an inactivated human immunodeficiency virus.

8. The method of claim 7 wherein said immunogen has been inactivated by removing a sufficient portion of its genetic material so as to render it incapable of replicating.

9. The method of claim 8 wherein the genetic material removed from said human immunodeficiency virus is a portion of a gene coding for a gag nucleocapsid protein.

10. The method of claim 7 wherein said human immunodeficiency virus has been inactivated by exposure to a solution of betapropiolactone.

11. The method of claim 1 wherein said immunogen is an infectious form of human immunodeficiency virus administered in a subinfectious amount.

12. The method of claim 1 wherein the effective amount of immunogen administered contains between 100 attograms and 20 milligrams of p24 gag antigen.

13. The method of claim 2 wherein the effective amount of immunogen administered contains between 10 and 500 femtograms of p24 gag antigen.

14. The method of claim 11 wherein the effective amount of immunogen administered contains between 100 attograms and 500 femtograms of p24 gaq antigen.

15. The method of claim 1 wherein a cell mediated response is determined to be present using a T-Cell proliferation assay if the uptake of thymidine by antigen-stimulated cells is at least four-fold above background.

16. The method of claim 1 wherein a cell mediated response is determined to be present using an IL-2 assay if the production of IL-2 by antigen-stimulated cells is at least four-fold above background.

17. A method for vaccinating a human against a human immunodeficiency virus comprising the steps of: selecting an immunogen competent to induce a protective immune response in said human against said human immunodeficiency virus, and administering an effective amount of said immunogen to said human sufficient to induce a cell mediated response against said human immunodeficiency virus but below the amount necessary to induce a humoral response.

18. A method for vaccinating a human against a mammalian retrovirus comprising the steps of: selecting an immunogen competent to induce a protective immune response in said mammal against said retrovirus, and administering an effective amount of said immunogen to said mammal sufficient to induce a cell mediated immune response against said retrovirus but below the level necessary to induce a humoral response.

19. The method of claim 18 wherein said retrovirus is a simian immunodeficiency virus.

20. The method of claim 18 wherein said mammal is a human.

21. The method of claim 20 wherein said retrovirus is HTLV-I.

22. The method of claim 20 wherein said retrovirus is HTLV-II.

23. The method of claim 20 wherein said retrovirus is foamy virus.

24. A vaccine comprising a therapeutically effective dose of an immunogen capable of eliciting a cell-mediated immune response in a human protective against infection by a human immunodeficiency virus.

25. A vaccine comprising a dose of immunogen capable of eliciting a

cell-mediated response in a human as measured by a T-cell proliferation assay.

L11 ANSWER 2 OF 19 USPATFULL
2002:238655 Retrovirus like particles made non infectious by a plurality of mutations.
Rovinski, Benjamin, Thornhill, CANADA
Cao, Shi-Xian, Etobicoke, CANADA
Yao, Fei-Long, North York, CANADA
Persson, Roy, North York, CANADA
Klein, Michel H., Willowdale, CANADA
Aventis Pasteur Limited, Toronto, CANADA (non-U.S. corporation)
US 6451322 B1 20020917
APPLICATION: US 1999-258128 19990226 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Non-infectious, retrovirus-like particles contain mutations to reduce gag-dependent RNA-packaging of the gag gene product, eliminate reverse transcriptase activity of the pol gene product, eliminate integrase activity of the pol gene, product and eliminate RNase H activity of the pol gene product through genetic manipulation of the gag and pol genes. The corresponding nucleic acid molecules are described. The non-infectious, retrovirus-like particles have utility in in vivo administration including to humans and in diagnosis.

CLM What is claimed is:
1. A non-infectious immunogenic, non-replicating human immunodeficiency virus (HIV)-like particle containing a plurality of mutations in the viral genome resulting in a virus-like particle, comprising the following: (1) a modified Gag protein, wherein said protein contains a modification, in the first Cys-His box only, wherein at least one amino acid residue has been replaced in said first Cys-His box, said replacement resulting in a reduction of gag-dependent genomic viral RNA packaging in the virus-like particle while retaining the immunogenicity of said virus-like particle; (2) a deficient reverse transcriptase, wherein said deficiency results from a deletion of that portion of the pot gene responsible for reverse transcriptase activity, said deletion substantially eliminating reverse transcriptase activity in the virus-like particle; (3) a deficient integrase, wherein said deficiency results from a deletion of that portion of the pol gene responsible for integrase activity, said deletion substantially eliminating integrase activity in the virus-like particle; and (4) a deficient RNase H, wherein the deficiency results from a deletion of that portion of the pot gene responsible for RNase H activity, said deletion substantially eliminating RNase H activity in the virus-like particle; wherein said particle is encoded by a modified HIV genome devoid of long terminal repeats (LTRs) and containing the gag, pol and env genes in their natural genomic arrangement, and additionally comprising at least one non-retroviral, non-mammalian heterologous antigenic marker wherein said particle encoded by said modified HIV genome deficient in long terminal repeats is further modified by a heterologous nucleic acid insert encoding said at least one antigenic marker and wherein said marker, when presented in the context of the retrovirus-like particle, is capable of generating an immune response specific to said antigenic marker when the particle is administered to a host.

2. The virus-like particle of claim 1, wherein said at least one antigenic marker is inserted into an insertion site located between

amino acid residues 210 and 211 of the gag gene product of the HIV-1 isolate LAI or the corresponding location of other retroviral gag gene products.

3. The virus-like particle of claim 2, wherein said at least one antigenic marker comprises from 1 to 4 tandem copies of the amino acid sequence AFDTRNRIIEVEN (SEQ ID:1).

4. A non-infectious, immunogenic, non-replicating human immunodeficiency virus (HIV)-like particle containing a plurality of mutations of the viral genome resulting in a virus-like particle comprising the following: (1) a modified Gag protein, wherein said protein contains a modification, in the first Cys-His box only, wherein at least one amino acid residue has been replaced in said first Cys-His box, said replacement resulting in a reduction of gag-dependent genomic viral RNA packaging in the virus-like particle while retaining the immunogenicity of said virus-like particle; (2) a deficient reverse transcriptase, wherein said deficiency results from a deletion of that portion of the pol gene responsible for reverse transcriptase activity, said deletion substantially eliminating reverse transcriptase activity in the virus-like particle; (3) a deficient integrase, wherein said deficiency results from a deletion of that portion of the pol gene responsible for integrase activity, said deletion substantially eliminating integrase activity in the virus-like particle; and (4) a deficient RNase H, wherein the deficiency results from a deletion of that portion of the pol gene responsible for RNase H activity, said deletion substantially eliminating RNase H activity in the virus-like particle; wherein said particle is encoded by a modified HIV genome devoid of long terminal repeats (LTRs) and containing the gag, pol and env genes in their natural genomic arrangement, and said virus-like particle further comprising a modified env gene product comprising a non-retroviral heterologous antigenic anchor sequence, wherein said heterologous anchor sequence replaces the endogenous anchoring functions of the env gene product wherein said particle is encoded by said modified HIV genome deficient in long terminal repeats is further modified by a heterologous nucleic acid insert encoding said heterologous antigenic anchor sequence and wherein said sequence, when generated in the context of the retrovirus-like particle, is capable of generating an immune response specific to said antigenic anchor sequence when the particle is administered to a host.

5. The virus-like particle of claim 4, wherein said anchor sequence is inserted into an insertion site of the env gene product adjacent to and upstream of functional cleavage sites of the env gene product.

6. The virus-like particle of claim 5, wherein said insertion site is located between amino acid residues 507 and 508 of the env gene product of the HIV-1 isolate LAI or the corresponding location of other retroviral env gene products.

7. The virus-like particle of claim 6, wherein the anchor sequence includes an amino acid sequence WILWISFAISCFLLCVVCWGSSCGPAKKATLGAT FAFDSKEEWCREKKEQWE (SEQ ID NO:4).

8. The virus-like particle of claim 6, wherein the anchor sequence includes an amino acid sequence WILWISFAISCFLLCVVLLGFIMW (SEQ ID NO:2).

9. The virus-like particle of claim 6, wherein the anchor sequence includes an amino acid sequence STVASSLALAIMIAGLSFWMCSNGSLQ (SED ID NO:3).

10. The virus-like particle of claim 1, wherein the human immunodeficiency virus is selected from the group consisting of HIV-1 and HIV-2.

11. An immunogenic composition capable of eliciting a retroviral specific immune response, comprising the virus-like particle of claim 1 or 4, and a carrier therefor.

12. The immunogenic composition of claim 11 formulated for mucosal or parenteral administration.

13. The immunogenic composition of claim 11 formulated for oral, anal, vaginal, or intranasal administration.

14. The immunogenic composition of claim 11 further comprising at least one other immunogenic and/or immunostimulating material.

15. The immunogenic composition of claim 14, wherein the at least one other immunostimulating material is an adjuvant.

16. The composition of claim 15, wherein the adjuvant is aluminum phosphate, aluminum hydroxide, Freund's incomplete adjuvant, or QS21.

17. The virus-like of claim 4 wherein the at least one amino acid is contained within amino acids Cys.sup.392 to Cys 395 of the gag gene product of the HIV-1 isolate LAI or the corresponding region of other HIV retroviral gag gene products.

18. The virus-like particles of claim 17 wherein Cys.sup.392 and/or Cys.sup.395 is replaced by serine.

19. The virus-like particles of claim 18 wherein both Cys.sup.392 and Cys.sup.395 are replaced by serine.

20. The virus-like particles of claim 4 wherein the at least a portion of the pol gene product contributing to reverse transcriptase activity is contained between amino acids Pro.sup.168 and Leu.sup.727 of the pot gene product of the HIV-1 isolate LAI or the corresponding region of other HIV retroviral pol gene products.

21. The virus-like particles of claim 4 wherein the at least a portion of the pol gene product contributing to integrase activity is contained between amino acids Phe.sup.728 and Asp.sup.1016 of the pol gene product of the HIV-1 isolate LAI or the corresponding region of other HIV retroviral pol gene products.

22. The virus-like particles of claim 4 wherein the substantial elimination of reverse transcriptase activity, integrase activity and RNase H activity all are substantially eliminated by deleting a portion of the pol gene product corresponding to amino acids Pro.sup.192 to Trp.sup.835 of the HIV-1 isolate LAI or the corresponding region of other HIV retroviral pol gene products.

23. The virus-like particles of claim 4 wherein the human immunodeficiency virus is selected from the group consisting of HIV-1 and HIV-2.

24. The virus-like particle of claim 1, wherein the at least one amino acid is contained within amino acids Cys392 to Cys395 of the gag gene product of the HIV-1 isolate LAI or the corresponding region of other HIV retroviral gag gene products.

25. The virus-like particle of claim 24, wherein Cys.sup.392 and/or Cys.sup.395 is replaced by serine.

26. The virus-like particle of claim 25, wherein both Cys.sup.392 and Cys.sup.395 are replaced by serine.

27. The virus-like particle of claim 1, wherein the at least a portion of the pol gene product contributing to reverse transcriptase activity is contained between amino acids Pro.sup.168 and Leu.sup.727 of the pot gene product of the HIV-1 isolate LAI or the corresponding region of other HIV retroviral pol gene products.

28. The virus-like particle of claim 1, wherein the at least a portion of the pol gene product contributing to integrase activity is contained between amino acids Phe.sup.728 and Asp.sup.1016 of the pol gene product of the HIV-1 isolate LAI or the corresponding region of other HIV retroviral pol gene products.

29. The virus-like particle of claim 1, wherein the substantial elimination of reverse transcriptase activity, integrase activity and RNase H activity all are substantially eliminated by deleting a portion of the pol gene product corresponding to amino acids Pro.sup.192 to Trp.sup.835 of the HIV-1 isolate LAI or the corresponding region of other HIV retroviral pol gene products.

L11 ANSWER 8 OF 19 USPATFULL
2000:80416 Human immunodeficiency virus type 1 nucleic acids devoid of long terminal repeats capable of encoding for non-infectious, immunogenic, retrovirus-like particles.
Rovinski, Benjamin, Thornhill, Canada
Cao, Shi-Xian, Etobicoke, Canada
Yao, Fei-Long, North York, Canada
Persson, Roy, North York, Canada
Klein, Michel H., Willowdale, Canada
Connaught Laboratories Limited, Toronto, Canada (non-U.S. corporation)
US 6080408 20000627
APPLICATION: US 1995-482810 19950607 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Non-infectious, retrovirus-like particles contain mutations to reduce gag-dependent RNA-packaging of the gag gene product, eliminate reverse transcriptase activity of the pol gene product, eliminate integrase activity of the pol gene product and eliminate RNase H activity of the pol gene product through genetic manipulation of the gag and pol genes. The corresponding nucleic acid molecules are described. The non-infectious, retrovirus-like particles have utility in diagnosis.

CLM What is claimed is:
1. A nucleic acid molecule, comprising: a modified human immunodeficiency virus (HIV) genome, deficient in long terminal repeats (LTRs), containing the gag, pol and env genes in their natural genomic arrangement and means for expression operatively connected to said modified HIV genome for production of gene products in cells to produce non-infectious, immunogenic, non-replicating HIV retrovirus-like particles comprising, in an assembly, the gag, pot and env gene products, wherein: (1) at least one codon in the gag gene has been mutated to effect modification to the gag gene

product in the first Cys-His box only of the gag gene product by replacing at least one amino acid residue in the first Cys-His box contributing to gag-dependent genomic viral RNA packaging into said HIV retrovirus-like particles to effect reduction of gag -dependent genomic viral RNA packaging into the particles while retaining the immunogenicity of the HIV retrovirus-like particles; (2) codons in the pol gene encoding a portion of the pol gene product contributing to reverse transcriptase activity have been deleted to substantially eliminate reverse transcriptase activity of the pol gene product in the HIV retrovirus-like particles; (3) codons in the pol gene encoding a portion of the pol gene product contributing to integrase activity have been deleted to substantially eliminate integrase activity of the pol gene product in the HIV retrovirus-like particles; and (4) codons in the pol gene encoding a portion of the pol gene product contributing to RNase H activity have been deleted to substantially eliminate RNase H activity of the pol gene product in the HIV retrovirus-like particles.

2. The nucleic acid molecule of claim 1 wherein said nucleic acid molecule comprises a SacI (678) to XhoI (8944) HIV-1.sub.LAI restriction fragment.

3. The nucleic acid molecule of claim 1, wherein said molecule is deficient in the primer binding site (PBS) and/or an RNA packaging signal.

4. The nucleic acid molecule of claim 1, wherein the reduction of gag-dependent RNA packaging is effected by mutagenesis of a region thereof encoding at least one amino acid contained within a region of the gag gene product corresponding to Cys.sup.392 to Cys.sup.395 of the HIV-1 LAI isolate, or a corresponding region of other HIV-1 isolates.

5. The nucleic acid molecule of claim 4, wherein a codon encoding Cys.sup.392 and/or Cys.sup.395 is replaced by a codon encoding serine.

6. The nucleic acid molecule of claim 5, wherein codons encoding both Cys.sup.392 and Cys.sup.395 are replaced by codons encoding serine.

7. The nucleic acid molecule of claim 1, wherein the HIV retrovirus is selected from the group consisting of HIV-1 and HIV-2.

8. The nucleic acid molecule of claim 1, wherein the env gene is an LAI env gene, an MN env gene or an env gene from a primary HIV-1 isolate.

9. An immunogenic composition capable of eliciting a retroviral specific immune response, comprising the nucleic acid molecule of claim 1 and a carrier therefor.

10. The immunogenic composition of claim 9 formulated for mucosal or parenteral administration.

11. The immunogenic composition of claim 9 formulated for oral, anal, vaginal, or intranasal administration.

12. The immunogenic composition of claim 9 further comprising at least one other immunogenic and/or immunostimulating material.

13. The immunogenic composition of claim 9, further comprising an adjuvant.

14. The composition of claim 13, wherein the adjuvant is aluminum phosphate, aluminum hydroxide, Freund's incomplete adjuvant, or QS21.

15. A method of immunizing a host to produce a retroviral specific immune response, comprising administering to the host an immunoeffective amount of the immunogenic composition of claim 9.

16. The nucleic acid molecule of claim 1, wherein the at least a part of the pol gene encoding reverse transcriptase is contained between nucleotides 2586 and 4265 of the pol gene of HIV-1 isolate LAI or the corresponding region of other HIV pol genes.

17. The nucleic acid molecule of claim 1, wherein the at least a part of the pol gene encoding integrase is contained between nucleotides 4266 and 5129 of the pol gene of HIV-1 isolate LAI or the corresponding region of other HIV pol genes.

18. The nucleic acid molecule of claim 1, wherein said modified retroviral genome includes a heterologous nucleic acid insert encoding at least one non-retroviral, non-mammalian antigenic marker, wherein said marker, when presented in the context of said HIV retrovirus-like particle, is capable of generating an immune response specific to said antigenic marker when the particle is administered to a host.

19. The nucleic acid molecule of claim 18, wherein the heterologous nucleic acid insert is inserted into the gag gene at the PstI site at nucleotide 1415 of the gag gene of HIV-1 LAI isolate or the corresponding location of other HIV gag genes.

20. The nucleic acid molecule of claim 19 wherein the heterologous nucleic acid insert comprises from 1 to 4 copies of a DNA sequence selected from the group consisting of: (a) 5' GCATTCGACACTAGAAATAGAATAATAGAAGTTGAAAAT 3' (SEQ ID NO:5); and (b) 3' CGTAAGCTGTGATCTTTATCTTATTATCTTCAACTTTTA 5' (SEQ ID NO: 6).

21. The nucleic acid molecule of claim 1 wherein said env gene has been modified to provide a modified env gene product in the HIV retrovirus-like particles in which endogenous anchoring function of env has been replaced by a non-retroviral antigenic anchor marker sequence operatively connected to the env gene product to anchor said env gene product to the HIV retrovirus-like particle, and wherein said marker, when presented in the context of the retrovirus-like product, is capable of generating an immune response specific to said antigenic marker when the particle is administered to a host.

22. The nucleic acid molecule of claim 21 wherein the antigenic anchor sequence comprises a DNA sequence selected from the group consisting of: (a) 5' TGGATCCTGTGGATTCCTTTGCCATATCATGCTTTTTGCTTTG TGTTGTTTTGCTGGGGTTCATCATGTGG 3' (SEQ ID NO: 7); and (b) 3' ACCTAGGACACCTAAAGGAAACGGTATAGTACGAAAAACGAAA CACAACAAAACGACCCCAAGTAGTACACC 5' (SEQ ID NO: 8).

23. The nucleic acid molecule of claim 21 wherein the antigenic anchor sequence includes a DNA sequence selected from the group consisting of: (a) 5' TCAACAGTGGCAAGTTCCCTAGCACTGGCAATCAT GATAGCTGTGCTATCTTTTTGGATGTGTT CCAATGGGTCATTGCAG 3' (SEQ ID NO: 9); and (b) 3' AGTTGTCACCGTTCAAGGGATCGTGACCGTTAGTACTATCGA CCAGATAGAAAAACCTACACAAGGTTACC CAGTAACGTC 5' (SEQ ID NO: 10).

24. The nucleic acid molecule of claim 21 wherein the antigenic anchor sequence includes a DNA sequence selected from the group consisting of:

(a) 5' TGGATCCTGTGGATTTCCTTTGCCATATCATGCTTTTTGC
TTTGTGTTGTTTGCTGGGGTTCATCATGTGGGCCTGCCAAAAAGGCA
ACGGTGCAACATTTGCATTTGATAGTAAAGAAGAGTGGTGCAGAGA GAAAAAAGAGCAGTGGGAA 3'
(SEQ ID NO. 11); and (b) 3' ACCTAGGACACCTAAAGGAAACGGTATAGTACGAAAAACG
AAACACAACAAACGACCCCAAGTAGTACACCCGGACGGTTTTTCCG
TTGAATCCACGTTGTAAACGTAAACTATCATTTCTTCTCACCACGTCT CTCTTTTTTCTCGTCACCCTT
5' (SEQ ID NO:12).

L17 ANSWER 30 OF 52 USPATFULL
2001:136438 Lentivirus-based gene transfer vectors.
Olsen, John C., Chapel Hill, NC, United States
The University of North Carolina at Chapel Hill, Chapel Hill, NC, United States (U.S. corporation)
US 6277633 B1 20010821
APPLICATION: US 1998-76707 19980512 (9)
PRIORITY: US 1997-46891P 19970513 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A recombinant lentiviral vector expression system comprises a first vector that comprises a nucleic acid sequence of at least part of the Equine Infectious Anemia Virus (EIAV) genome. The vector contains at least one defect in at least one gene encoding an EIAV structural protein, but is preferably a gaglpol expression vector. The expression system further comprises a second vector, also comprising a nucleic acid sequence of at least part of the Equine Infectious Anemia Virus (EIAV) genome, and additionally containing a multiple cloning site wherein a heterologous gene may be inserted. The expression system also comprises a third vector which expresses a viral envelope protein. The first and third vectors are packaging signal-defective. When the expression system is transfected into a lentivirus-permissive cell, replication-defective EIAV particles may be produced, which particles are useful in delivering heterologous genes to a broad range of both dividing and non-dividing cells.

CLM What is claimed is:
1. A recombinant lentiviral vector expression system comprising: (a) a first vector comprising an Equine Infectious Anemia Virus (EIAV) nucleic acid sequence encoding EIAV gag and EIAV pol, wherein said vector (i) comprises at least one defect in at least one gene encoding an EIAV structural protein, and (ii) comprises a defective packaging signal; (b) a second vector comprising an EIAV nucleic acid sequence comprising cis-acting sequence elements required for reverse transcription of the vector genome, wherein said vector (i) comprises a competent packaging signal, and (ii) comprises a multiple cloning site wherein a heterologous gene may be inserted; and (c) a third vector comprising a viral nucleic acid sequence, wherein said third vector (i) expresses a viral envelope protein, and (ii) comprises a defective packaging signal.

2. A vector system according to claim 1, wherein said second vector is deficient for expression of at least one EIAV structural protein.

3. A vector system according to claim 1, wherein said first vector, said second vector, and said third vector are obtained from cDNA clones of the EIAV genome.

4. A vector expression system according to claim 1, wherein said first vector is a gag-pol expression vector, and wherein said vector comprises a defect in the env gene.

5. A vector expression system according to claim 4, wherein said defect in the env gene is a deletion mutation.

6. A vector expression system according to claim 1, wherein said first vector and said second vector each comprise a defect in the env gene.

7. A vector expression system according to claim 1, wherein said third vector encodes an envelope protein that is not an EIAV envelope protein.

8. A vector expression system according to claim 1, wherein said third vector expresses the vesicular stomatitis virus G glycoprotein.

9. A vector expression system according to claim 1, wherein said second vector comprises a heterologous gene.

10. A vector expression system according to claim 9, wherein said heterologous gene encodes an antigenic protein or peptide.

11. A vector expression system according to claim 1, wherein said first vector is selected from the group consisting of the plasmid pEV53 and the plasmid pEV53A; said second vector is selected from the group consisting of pEC-lacZ and pEC-puro; and said third vector is the plasmid pCI-VSV-G.

12. The plasmid set forth in FIG. 2A as pEV53.

13. The plasmid set forth in FIG. 2B as pEV53A.

14. The plasmid set forth in FIG. 2 as pEC lacZ.

15. The plasmid set forth in FIG. 3 as pEC-puro.

16. The plasmid set forth in FIG. 4 as pCI-VSV-G.

17. A method of producing a replication-defective lentivirus particle, comprising transfecting a cell with: (a) a first vector comprising an Equine Infectious Anemia Virus (EIAV) nucleic acid sequence encoding EIAV gag and EIAV pol, wherein said vector (i) comprises at least one defect in at least one gene encoding an EIAV structural protein, and (ii) comprises a defective packaging signal; (b) a second vector comprising an EIAV nucleic acid sequence comprising cis-acting sequence elements required for reverse transcription of the vector genome wherein said vector (i) comprises a competent packaging signal, and (ii) comprises a multiple cloning site wherein a heterologous gene may be inserted; and (c) a third vector comprising a nucleic acid sequence of a virus, wherein said third vector (i) expresses a viral envelope protein, and (ii) comprises a defective packaging signal, wherein the cell produces a replication-defective lentivirus particle.

18. A method according to claim 17, wherein said cell is a non-dividing

cell.

19. A method according to claim 17, wherein said second vector comprises a heterologous gene.

20. A replication-defective lentivirus particle produced according to the method of claim 17.

21. A cell comprising a replication-defective lentiviral particle, wherein said lentiviral particle is produced according to the method of claim 17.

22. An infectious EIAV particle comprising a nucleic acid sequence encoding a promoter and a gene sequence heterologous to EIAV, and wherein said nucleic acid sequence is defective in encoding at least one EIAV structural protein so that said virus particle is replication defective.

23. A method of producing a lentiviral stock comprising: (a) transfecting a lentivirus-permissive cell with (i) a first vector comprising an Equine Infectious Anemia Virus (EIAV) nucleic acid sequence encoding EIAV gag and EIAV pol, wherein said vector (1) comprises at least one defect in at least one gene encoding an EIAV structural protein, and (2) comprises a defective packaging signal; (ii) a second vector comprising an EIAV nucleic acid sequence comprising cis-acting sequence elements required for reverse transcription of the vector genome wherein said vector (1) comprises a competent packaging signal, (2) comprises a heterologous gene; and (iii) a third vector comprising a nucleic acid sequence of a virus, wherein said third vector (1) expresses a viral envelope protein, and (2) comprises a defective packaging signal; (b) growing the cell under cell culture conditions sufficient to allow production of replication-defective lentivirus particles in the cell; and (c) collecting said replication-defective lentivirus particles from the cell.

24. A method according to claim 23, wherein said producer cell is grown in a cell culture medium, and wherein said replication-defective lentivirus particles are collected from said medium.

25. A method of making a packaging cell, comprising transfecting a lentivirus-permissive cell with a vector comprising an EIAV nucleic acid sequence, wherein said vector comprises a defective packaging signal.

26. A method according to claim 25, wherein said vector is a gag-pol expression vector.

27. A method according to claim 25, wherein said vector is selected from the group consisting of the plasmid pEV53 and the plasmid pEV53 A.

28. A method according to claim 25, wherein said lentivirus-permissive cell is a human 293 cell.

29. A packaging cell comprising a lentivirus-permissive host cell comprising an EIAV nucleic acid sequence encoding at least one EIAV structural protein, wherein said nucleic acid sequence is packaging-signal defective, such that the cell itself produces at least one EIAV structural protein, but does not produce

replication-competent infectious virus.

L17 ANSWER 27 OF 52 USPATFULL
2001:196592 Equine infectious anemia virus vectors.
Kingsman, Alan John, Oxon, United Kingdom
Carroll, Miles William, Oxon, United Kingdom
Rohll, Jonathan, Berkshire, United Kingdom
Mitrophanous, Kyriacos, Oxford, United Kingdom
Kim, Narry, Seoul, Korea, Republic of
Oxford Biomedica (UK) Limited, Oxford, United Kingdom (non-U.S. corporation)
US 6312683 B1 20011106
APPLICATION: US 1999-238356 19990127 (9)
PRIORITY: GB 1997-27135 19971222
GB 1998-11037 19980522
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A retroviral vector derived from a non-primate lentivirus genome comprising a deleted gag gene wherein the deletion in gag removes one or more nucleotides downstream of nucleotide 350 of the gag coding sequence.

CLM What is claimed is:
1. An equine infectious anemia virus (EIAV) vector comprising a gag gene nucleotide coding sequence that is modified so as to contain a deletion located at least 354 nucleotides downstream from the ATG initiation codon of the gag gene in EIAV.

2. The EIAV vector according to claim 1 wherein the deletion is from nucleotide 354 to at least the C-terminus of the gag-pol coding region in EIAV.

3. The EIAV vector according to claim 1 wherein the deletion additionally removes nucleotide 300 of the gag gene nucleotide coding sequence in EIAV.

4. The EIAV vector according to any one of claims 1, 2, or 3 wherein the deletion further includes at least one nucleotide upstream from the nucleotide at position 354 downstream from the ATG initiation codon of the gag gene in EIAV, and wherein the vector retains the first 150 nucleotides of the gag gene nucleotide coding sequence in EIAV.

5. The EIAV vector according to any one of claims 1, 2, or 3 wherein the deletion further includes at least one nucleotide upstream from the nucleotide at position 354 downstream from the ATG initiation codon of the gag gene in EIAV, and wherein the vector retains the first 109 nucleotides of the gag gene coding nucleotide sequence in EIAV.

6. The EIAV vector according to any one of claims 1, 2, or 3 wherein the deletion further includes at least one nucleotide upstream from the nucleotide at position 354 downstream from the ATG initiation codon of the gag gene in EIAV, and wherein the vector retains the first 2 nucleotides of the gag gene nucleotide coding sequence in EIAV.

7. The EIAV vector according to any one of claims 1, 2 or 3 wherein one or more accessory genes are absent from the

vector.

8. The EIAV vector according to claim 7 wherein the accessory genes are selected from the group consisting of dUTPase, S2, rev and tat, and combinations thereof.

9. The EIAV vector according to claim 4 wherein the EIAV gag gene coding sequence is only the first 150 nucleotides of the gag gene coding sequence in EIAV.

10. The EIAV vector according to claim 5 wherein the EIAV gag gene coding sequence is only the first 109 nucleotides of the gag gene coding sequence in EIAV.

11. The EIAV vector according to claim 6 wherein the EIAV gag gene coding sequence is only the first 2 nucleotides of the gag gene coding sequence in EIAV.

12. A method for expressing a gene product comprising contacting a cell with the EIAV vector of any one of claims 1, 2 or 3, wherein the vector includes a nucleic acid sequence encoding said gene product.

13. An isolated cell comprising the EIAV vector according to any one of claims 1, 2 or 3.

14. A delivery system comprising the EIAV vector according to any one of claims 1, 2 or 3, and a pharmaceutically acceptable carrier.

15. The EIAV vector according to any one of claims 1, 2 or 3 further comprising a heterologous nucleotide sequence of interest.

16. An isolated equine infectious anemia virus (EIAV)-based vector particle comprising functionally active gag-pol proteins, and further comprising a nucleic acid sequence of EIAV gag nucleotide residues having a deletion located at least 350 nucleotides downstream from the ATG initiation codon of the EIAV gag coding sequence.

17. The EIAV vector according to claim 2 wherein the deletion additionally removes nucleotide 300 of the gag gene nucleotide coding sequence in EIAV.

18. The EIAV vector according to claim 4 wherein one or more accessory genes are absent from the vector.

19. The EIAV vector according to claim 18 wherein the accessory genes are selected from the group consisting of dUTPase, S2, rev and tat, and combinations thereof.

20. The EIAV vector according to claim 5 wherein one or more accessory genes are absent from the vector.

21. The EIAV vector according to claim 20 wherein the accessory genes are selected from the group consisting of dUTPase, S2, rev and tat, and combinations thereof.

22. The EIAV vector according to claim 6 wherein one or more accessory genes are absent from the vector.

23. The EIAV vector according to claim 22 wherein the accessory genes are selected from the group consisting of dUTPase, S2, rev and tat, and combinations thereof.

24. An equine infectious anemia virus (EIAV)-based retroviral vector particle comprising a gag gene nucleotide coding sequence that is modified so as to contain a deletion located at least 354 nucleotides downstream from the ATG initiation codon of the gag gene in EIAV.

25. The EIAV-based retroviral vector particle according to claim 24 wherein the deletion is from nucleotide 354 to at least the C-terminus of the gag-pol coding region in EIAV.

26. The EIAV-based retroviral vector particle according to claim 24 wherein the deletion additionally removes nucleotide 300 of the gag gene nucleotide coding sequence in EIAV.

27. The EIAV-based retroviral vector particle according to any one of claims 24, 25, or 26 wherein the deletion further includes at least one nucleotide upstream from the nucleotide at position 354 downstream from the ATG initiation codon of the gag gene in EIAV, and wherein the vector particle retains the first 150 nucleotides of the gag gene nucleotide coding sequence in EIAV.

28. A production system for producing the EIAV-based retroviral vector particle of any one of claims 24, 25 or 26 comprising a packaging cell comprising an EIAV-based RNA genome, a gag-pol gene, and an envelope gene, wherein said genome comprises a gag gene nucleotide coding sequence that is modified so as to contain a deletion located at least 354 nucleotides downstream from the ATG initiation codon of the gag gene nucleotide coding sequence in EIAV.

29. An EIAV-based retroviral vector particle produced by the production system of claim 28.

30. The EIAV-based retroviral vector particle according to any one of claims 24, 25, or 26 wherein the deletion further includes at least one nucleotide upstream from the nucleotide at position 354 downstream from the ATG initiation codon of the gag gene in EIAV, and wherein the vector particle retains the first 109 nucleotides of the gag gene nucleotide coding sequence in EIAV.

31. The EIAV-based retroviral vector particle according to any one of claims 24, 25, or 26 wherein the deletion further includes at least one nucleotide upstream from the nucleotide at position 354 downstream from the ATG initiation codon of the gag gene in EIAV, and wherein the vector particle retains the first 2 nucleotides of the gag gene nucleotide coding sequence in EIAV.

32. The EIAV-based retroviral vector particle according to any one of claims 24, 25 or 26 wherein one or more accessory genes or gene products therefrom are absent from the vector particle.

33. The EIAV-based retroviral vector particle according to claim 32 wherein the accessory genes are selected from the group consisting of dUTPase, S2, rev and tat, and combinations thereof.

34. The EIAV-based retroviral vector particle according to claim 27 wherein the EIAV gag gene coding sequence is only the first 150 nucleotides of the gag gene coding sequence in EIAV.

35. The EIAV-based retroviral vector particle according to claim 30 wherein the EIAV gag gene coding sequence is only the first 109 nucleotides of the gag gene coding sequence in EIAV.

36. The EIAV-based retroviral vector particle according to claim 31 wherein the EIAV gag gene coding sequence is only the first 2 nucleotides of the gag gene coding sequence in EIAV.

37. The EIAV-based retroviral vector particle according to claim 25 wherein the deletion additionally removes nucleotide 300 of the gag gene nucleotide coding sequence in EIAV.

38. The EIAV-based retroviral vector particle according to claim 27 wherein one or more accessory genes or gene products therefrom are absent from the vector particle.

39. The EIAV-based retroviral vector particle according to claim 38 wherein the accessory genes are selected from the group consisting of dUTPase, S2, rev and tat, and combinations thereof.

40. The EIAV-based retroviral vector particle according to claim 30 wherein one or more accessory genes or gene products therefrom are absent from the vector particle.

41. The EIAV-based retroviral vector particle according to claim 40 wherein the accessory genes are selected from the group consisting of dUTPase, S2, rev and tat, and combinations thereof.

42. The EIAV-based retroviral vector particle according to claim 31 wherein one or more accessory genes or gene products therefrom are absent from the vector particle.

43. The EIAV-based retroviral vector particle according to claim 42 wherein the accessory genes are selected from the group consisting of dUTPase, S2, rev and tat, and combinations thereof.

44. A method for expressing a gene product comprising contacting a cell with the EIAV-based retroviral vector particle of any one of claims 24, 25 or 26, wherein the vector particle includes a nucleic acid sequence encoding said gene product.

45. An isolated cell comprising the EIAV-based retroviral vector particle according to any one of claims 24, 25 or 26.

46. A delivery system comprising the EIAV-based retroviral vector particle according to any one of claims 24, 25 or 26, and a pharmaceutically acceptable carrier.

47. The EIAV-based retroviral vector particle according to any one of claims 24, 25 or 26 further comprising a heterologous nucleotide sequence of interest.

48. The EIAV-based retroviral particle according to claim 32 further comprising a heterologous nucleotide sequence of interest.

49. The EIAV-based retroviral particle according to claim 33 further comprising a heterologous nucleotide sequence of interest.

50. The EIAV vector according to claim 7 further comprising a heterologous nucleotide sequence of interest.

51. The EIAV vector according to claim 8 further comprising a heterologous nucleotide sequence of interest.

52. The production system of claim 28 further comprising a heterologous nucleotide sequence of interest.

53. The production system of claim 28 wherein one or more accessory genes or gene products thereof are absent.

54. The method of claim 53 wherein the accessory genes are selected from the group consisting of dUTPase, S2, rev and tat, and combinations thereof.

55. A method for producing the EIAV-based retroviral vector particle of any one of claims 24, 25 or 26 comprising co-expressing in a packaging cell nucleic acid sequence(s) encoding an EIAV-based RNA genome, a gag-pol gene, and an envelope gene, wherein said genome comprises a gag gene nucleotide sequence that is modified so as to contain a deletion located at least 354 nucleotides downstream from the ATG initiation codon of the gag gene in EIAV.

56. The method of claim 55 wherein the genome includes a heterologous nucleotide sequence of interest.

57. The method of claim 56 wherein one or more accessory genes or gene products thereof are absent.

58. The method of claim 57 wherein the accessory genes are selected from the group consisting of dUTPase, S2, rev and tat, and combinations thereof.

59. A production system for producing the EIAV-based retroviral vector particle of any one of claim 16 comprising a packaging cell comprising an EIAV-based RNA genome, a gag-pol gene, and an envelope gene, wherein said genome comprises a nucleic acid sequence of EIAV gag nucleotide residues having a deletion located at least 350 nucleotides downstream from the ATG initiation codon of the EIAV gag coding sequence.

60. An EIAV-based retroviral vector particle produced by the production system of claim 59.

61. A method for producing the EIAV-based retroviral vector particle of claim 16 comprising co-expressing in a packaging cell nucleic acid sequence(s) encoding an EIAV-based RNA genome, a gag-pol gene, and an envelope gene, wherein said

genome comprises a nucleic acid sequence of EIAV gag nucleotide nucleotide residues having a deletion located at least 350 nucleotides downstream from the ATG initiation codon of the EIAV gag gene coding sequence.

62. The method of claim 61 wherein the genome includes a heterologous nucleotide sequence of interest.

63. The method of claim 62 wherein one or more accessory genes or gene products thereof are absent.

64. The method of claim 63 wherein the accessory genes are selected from the group consisting of dUTPase, S2, rev and tat, and combinations thereof.

L18 ANSWER 3 OF 4 WPIDS (C) 2003 THOMSON DERWENT
AN 1996-117413 [13] WPIDS
DNC C1996-037266
TI Vaccination of humans against HIV - by admin. of an immunogen which induces a sustained cell-mediated immune response against HIV but does not activate a humoral response.
DC B04 D16
IN BENVENISTE, R E; CLERICI, M S; SHEARER, G M
PA (USSH) US DEPT HEALTH & HUMAN SERVICES
CYC 1
PI CA 2124545 A 19951128 (199613)* EN 33p
ADT CA 2124545 A CA 1994-2124545 19940527
PRAI CA 1994-2124545 19940527

AB CA 2124545 A UPAB: 19960329
Humans are vaccinated against HIV by admin. of an immunogen to induce a sustained cell-mediated immune response against HIV. Also claimed are: (1) a method for vaccinating a human against a mammalian retrovirus by admin. of an amt. of immunogen sufficient to induce a cell-mediated immune response against the retrovirus but below the level necessary to induce a humoral response; and (2) a vaccine comprising an immunogen which elicits a cell-mediated immune response in a human to protect against HIV infection.

USE - The method and vaccine are useful to induce protection against HIV in humans (claimed). They activate a protective cell-mediated response, but avoid reducing this response through the activation of an offsetting humoral response. The method can also be used to induce a protective immune response against other retroviruses, partic. SIV, HTLV-I or II, or foamy virus.
Dwg.0/1

L2 ANSWER 3 OF 37 MEDLINE
1999119499 Document Number: 99119499. PubMed ID: 9918884. Nucleocapsid protein zinc-finger mutants of simian immunodeficiency virus strain mne produce virions that are replication defective in vitro and in vivo. Gorelick R J; Benveniste R E; Gagliardi T D; Wiltrout T A; Busch L K; Bosche W J; Coren L V; Lifson J D; Bradley P J; Henderson L E; Arthur L O. (AIDS Vaccine Program, SAIC-Frederick, Frederick Cancer Research and Development Center, SAIC-Frederick, Frederick, Maryland, 21702-1201, USA.. gorelick@avpaxp1.ncicrf.gov) . VIROLOGY, (1999 Jan 20) 253 (2) 259-70. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB All retroviruses (except the spumaretroviruses) contain a nucleocapsid (NC) protein that encodes one or two copies of the Zn2+-finger sequence -Cys-X2-Cys-X4-His-X4-Cys-. This region has been shown to be essential for recognition and packaging of the genomic RNA during virion particle assembly. Additionally, this region has been shown to be involved in early infection events in a wide spectrum of retroviruses, including mammalian type C [e.g., murine leukemia virus (MuLV)], human immunodeficiency virus type 1 (HIV-1), Rous sarcoma virus, and other retroviruses. Mutations in the two Zn2+-fingers of the NC protein of simian immunodeficiency virus strain Mne [SIV(Mne)] have been generated. The resulting virions contained the normal complement of processed viral proteins with densities indistinguishable from wild-type SIV(Mne). All of the mutants had electron micrograph morphologies similar to those of immature particles observed in wild-type preparations. RNA packaging was less affected by mutations in the NC protein of SIV(Mne) than has been observed for similar mutants in the MuLV and HIV-1 systems. Nevertheless, in vitro replication of SIV(Mne) NC mutants was impaired to levels comparable to those observed for MuLV and HIV-1 NC mutants; replication defective NC mutants are typically 10(5)- to 10(6)-fold less infectious than similar levels of wild-type virus. One mutant, DeltaCys33-Cys36, was also found to be noninfectious in vivo when mutant virus was administered intravenously to a pig-tailed macaque. NC mutations can therefore be used to generate replication defective virions for candidate vaccines in the SIV macaque model for primate lentiviral diseases.

L3 ANSWER 2 OF 4 MEDLINE
2001083055 Document Number: 20541994. PubMed ID: 11090194. Protection of Macaca nemestrina from disease following pathogenic simian immunodeficiency virus (SIV) challenge: utilization of SIV nucleocapsid mutant DNA vaccines with and without an SIV protein boost. Gorelick R J; Benveniste R E; Lifson J D; Yovandich J L; Morton W R; Kuller L; Flynn B M; Fisher B A; Rossio J L; Piatak M Jr; Bess J W Jr; Henderson L E; Arthur L O. (AIDS Vaccine Program, SAIC-Frederick, Frederick Cancer Research and Development Center, Frederick, Maryland 21702-1201, USA.) JOURNAL OF VIROLOGY, (2000 Dec) 74 (24) 11935-49. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Molecular clones were constructed that express nucleocapsid (NC) deletion mutant simian immunodeficiency viruses (SIVs) that are replication defective but capable of completing virtually all of the steps of a single viral infection cycle. These steps include production of particles that are viral RNA deficient yet contain a full complement of processed viral proteins. The mutant particles are ultrastructurally indistinguishable from wild-type virus. Similar to a live attenuated vaccine, this approach should allow immunological presentation of a full range of viral epitopes, without the safety risks of replicating virus. A

total of 11 Macaca nemestrina macaques were inoculated with NC mutant SIV expressing DNA, intramuscularly (i.m.) in one study and i.m. and subcutaneously in another study. Six control animals received vector DNA lacking SIV sequences. Only modest and inconsistent humoral responses and no cellular immune responses were observed prior to challenge. Following intravenous challenge with 20 animal infectious doses of the pathogenic SIV(Mne) in a long-term study, all control animals became infected and three of four animals developed progressive SIV disease leading to death. All 11 NC mutant SIV DNA-immunized animals became infected following challenge but typically showed decreased initial peak plasma SIV RNA levels compared to those of control animals ($P = 0.0007$). In the long-term study, most of the immunized animals had low or undetectable postacute levels of plasma SIV RNA, and no CD4(+) T-cell depletion or clinical evidence of progressive disease, over more than 2 years of observation. Although a subset of immunized and control animals were boosted with SIV(Mne) proteins, no apparent protective benefit was observed. Immunization of macaques with DNA that codes for replication-defective but structurally complete virions appears to protect from or at least delay the onset of AIDS after infection with a pathogenic immunodeficiency virus. With further optimization, this may be a promising approach for vaccine development.

L3 ANSWER 1 OF 4 MEDLINE
2001323771 Document Number: 20536033. PubMed ID: 11085583. Mucosal challenge of Macaca nemestrina with simian immunodeficiency virus (SIV) following SIV nucleocapsid mutant DNA vaccination. Gorelick R J; Lifson J D; Yovandich J L; Rossio J L; Piatak M Jr; Scarzello A J; Knott W B; Bess J W Jr; Fisher B A; Flynn B M; Henderson L E; Arthur L O; Benveniste R E. (AIDS Vaccine program, SAIC-Frederick, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, MD 21702-1201, USA.) JOURNAL OF MEDICAL PRIMATOLOGY, (2000 Aug) 29 (3-4) 209-19. Journal code: 0320626. ISSN: 0047-2565. Pub. country: Denmark. Language: English.

AB A simian immunodeficiency virus (SIV)(Mne) DNA clone was constructed that produces viruses containing a four amino acid deletion in the second zinc finger of the nucleocapsid (NC) domain of the Gag polyprotein. Viruses produced from this clone, although non-infectious both in vitro and in vivo, complete a majority of the steps in a single retroviral infection cycle. Eight pig-tailed macaques (Macaca nemestrina) were inoculated intramuscularly and subcutaneously three times over the course of 24 weeks with the NC mutant expressing DNA. These macaques, and four controls, were then challenged mucosally (intrarectally) with the homologous virus (SIV Mne CL E11S) and monitored for evidence of infection and clinical disease. Prior to challenge, a measurable humoral immune response was noted in four of eight immunized macaques. After challenge, all 12 macaques became infected, although four immunized animals greatly restricted their viral replication, and one immunized animal that controlled replication remains antibody negative. No disease has been evidence during the 46-week period of monitoring after challenge.

L9 ANSWER 9 OF 19 MEDLINE
97235597 Document Number: 97235597. PubMed ID: 9080716. Vaccine strategies: selective elicitation of cellular or humoral immunity?. Shearer G M; Clerici M. (Experimental Immunology Branch, National Cancer Institute, national Institutes of Health, Bethesda, MD 20892, USA.. shearerg@dc10a.nci.nih.gov) . TRENDS IN BIOTECHNOLOGY, (1997 Mar) 15 (3) 106-9. Ref: 19. Journal code: 8310903. ISSN: 0167-7799. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The discovery of immunoregulatory cytokines and the fact that they modulate the cellular and humoral arms of the immune system, generally in opposing directions raises fundamental questions concerning vaccine development. Because antibiotic-resistance infectious organisms are appearing at an increasingly rapid rate, more emphasis will need to be placed on prevention of infection/disease via immunization and less on post-infection antibiotics. To accomplish this task effectively, immune regulation should be integrated into vaccine design. Here we consider the opposing potentials of cytokine-regulated cellular and humoral immunity, and question whether the "best of both worlds' is possible or desirable.

L16 ANSWER 27 OF 28 MEDLINE
90219187 Document Number: 90219187. PubMed ID: 2109098. Mutations of RNA and protein sequences involved in human immunodeficiency virus type 1 packaging result in production of noninfectious virus. Aldovini A; Young R A. (Whitehead Institute for Biomedical Research, Nine Cambridge Center, Massachusetts 02142.) JOURNAL OF VIROLOGY, (1990 May) 64 (5) 1920-6. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB To identify RNA and protein sequences involved in packaging of human immunodeficiency virus type 1 (HIV-1), various mutations were introduced into the viral genome. Portions of the human immunodeficiency virus type 1 genome between the first splice donor site and the gag initiation codon were deleted to investigate the RNA packaging site (psi). Point mutations that alter cysteine residues in one or both zinc finger motifs of p7, a cleavage product of the gag precursor, were created to study the role of the gag zinc fingers in packaging. The psi site mutants and the gag mutants exhibited similar phenotypes. Cells transfected with the mutant genomes, while expressing normal levels of human immunodeficiency virus type 1 RNA and proteins, produced viral particles that were normal in protein content but lacked detectable viral RNA. These mutant virions were unable to productively infect cells. The combination of human immunodeficiency virus type 1 packaging mutations should minimize fortuitous assembly of infectious virus and may provide a means to produce noninfectious particles for candidate vaccines.

L16 ANSWER 26 OF 28 MEDLINE
90279048 Document Number: 90279048. PubMed ID: 2191147. Noninfectious human immunodeficiency virus type 1 mutants deficient in genomic RNA. Gorelick R J; Nigida S M Jr; Bess J W Jr; Arthur L O; Henderson L E; Rein A. (Laboratory of Molecular Virology and Carcinogenesis, National Cancer Institute-Frederick Cancer Research Facility, Maryland 21701-1013.) JOURNAL OF VIROLOGY, (1990 Jul) 64 (7) 3207-11. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB All retroviruses contain, in the nucleocapsid domain of the Gag protein, one or two copies of the sequence Cys-X2-Cys-X4-His-X4-Cys. We have generated a series of mutants in the two copies of this motif present in human immunodeficiency virus type 1. These mutants encoded virus particles that were apparently composed of the normal complement of viral proteins but contained only 2 to 20% of the normal level of genomic RNA. No infectivity could be detected in the mutant particles, while 10(5) infectious U were present in an equivalent amount of wild-type particles. Thus, the mutants have another defect in addition to the inefficiency with which they encapsidate genomic RNA. Our results show that both copies of the motif are required for normal RNA

packaging and for infectivity. Mutants of this type may have important applications, including nonhazardous materials for research, immunogens in vaccine and immunotherapy studies, and diagnostic reagents.

L16 ANSWER 9 OF 28 MEDLINE
2000465940 Document Number: 20473930. PubMed ID: 11017793. DNA vaccination of macaques by a full genome HIV-1 plasmid which produces noninfectious virus particles. Akahata W; Ido E; Shimada T; Katsuyama K; Yamamoto H; Uesaka H; Ui M; Kuwata T; Takahashi H; Hayami M. (Laboratory of Viral Pathogenesis, Institute for Virus Research, Kyoto 606-8507, Japan.) VIROLOGY, (2000 Sep 15) 275 (1) 116-24. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB In this study, we tried a DNA vaccination regime in rhesus macaques using a full genome HIV-1 plasmid. The HIV-1 genome is under the control of its original LTR promoter, but has a mutated zinc finger motif gene in the nucleocapsid region. Due to the lack of genomic RNA packaging, the plasmid produces only noninfectious viral particles. We repeatedly injected four macaque monkeys intramuscularly with the naked DNA over a period of 40 weeks. To evaluate the humoral and cell-mediated immunity provided by this DNA vaccination, no other booster or other recombinant viral vectors were used. Immunological responses against HIV-1 were elicited in all of the vaccinated monkeys: stable anti-HIV-1 Env antibodies were raised in two monkeys and CTL activities were induced in the other monkeys. The macaques were intravenously challenged at 54 weeks with 100 TCID(50) of SHIV-NM-3rN, which possesses an envelope gene homologous to the one in the vaccinated plasmid. In all of the vaccinated macaques, the peak plasma viral loads induced by the challenge virus were two to three orders of magnitude lower than those of the naive controls. These results suggest that a DNA vaccination regime with a full genome plasmid alone is potentially efficacious and provides a new possibility for the development of an AIDS vaccine.